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THE BACTERIOLOGICAL METABOLISM OF GLYCEROL AND ITS DERIVATIVES

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I. There is no point in entering upon a lengthy exposition of the history of glycerol in bacteriology. Ever since the very earliest studies, this trivalent alcohol has been used in preparing cultures; its flexibility has rendered it invaluable both in its destructive phase and in bacterial synthesis. The enormous literature on glycerol indicates its almost universal utilization by both pathogenic and non-pathogenic bacteria, as well as by the sugar moulds and other fungi.

The data on the phosphoric esters of glycerol, on the other hand, are rather scant: Quastel and Woodbridge (1927, I and II), studied the anaerobe reduction of methylene blue by E. coli in the presence of very numerous substrata which included glycerol, its alpha and beta phosphates, and ethylenic glycol. Under their experimental conditions, the alpha phosphate is the most active source of hydrogen; the beta phosphate is less active, while the non-esterized glycerol is the least active, if not totally inactive source. The de-hydrogenating capacity of E. coli upon the alpha phosphate of glycerol is markedly greater under non-optimal conditions of temperature and pH, and in conjunction with certain inhibitors. According to this data, glycol can be a good source of hydrogen at the optimal temperature of 37°C.

In 1935, Tikka, working with E. coli, found that glycerol and its alpha phosphate yielded ethyl alcohol, formic acid, CO_2 and H_2. This did not occur with the beta phosphate.

In 1938, Wood, Wiggert and Werkmann studied the selective capacity of Propionibacterium pentosaceum in utilizing glycerol and its phosphoric alpha ester. In 1941, Quastel and Webley, working with propionic acid

bacteria, noted that glycerol is more readily utilized than its alpha phosphate, and that, in any case, the consumption of O_2 rises in the presence of Thyamin.

The very recent research (1946) conducted by Tosic with an Acetobacter shows that this germ can oxidize glycerol at a very great rate, but cannot affect either the alpha or beta esters.

Data on the other glycerol derivatives are extremely sparse. We shall have something to say about them in the body of this essay.

In an earlier paper, (1946) we reported the varying capacity of several germs to utilize glycerol and its alpha and beta phosphates in aerobiosis. This preliminary research shows that almost all the germs we used (both then and during the research that is the subject of this paper) oxidized glycerol and its alpha phosphate fairly well, but that, under our experimental conditions (the resting phase, pH 7.4, 38°C) they had very little effect on the beta phosphate of glycerol.

O_2 consumption in the presence of glycerol is twice that utilized in oxidation of the alpha phosphate, which may well lead us to conclude that for every quantity of O_2 the same quantity of substratum is consumed, keeping in mind the fact that only one of the synthetic alpha phosphates of glycerol (which also proves to be levogyrous) is biologically activated as occurs in animal tissues. (Cfr. Elliott, 1941). It should also be remembered that our research was conducted in the presence of buffering phosphates, and that therefore the glycerine oxidation might quite likely occur after phosphorilization, or, in other words, via a transition through a biologically active alpha phosphate of glycerol.

~~Therefore, this intensive~~ Previous research showed that ~~in any case,~~ oxidation of the glycerol molecule, phosphatized or not, could occur only in the presence of two alcoholic functions. ~~For that matter,~~ data already available in the literature also indicated that when only one hydroxide is present, for example in the propane molecule, if it is a primary hydroxide, it can be oxidized by several species of bacteria, forming propionic acid. But, in the case of a secondary hyrdoxide (isopropyl alcohol), only a few species can successfully attack it, and they are usually the acetobacter group (~~Bernhauer, 1940~~).

Furthermore, before beginning this project, we wanted to find out whether, under ~~our~~ experimental conditions (with the germs in the resting phase), there were similar behavior in the presence of the monovalent alcohols of propane (propyl and isopropyl) on the part of germs such as E. coli, Ps. aeruginosa, and B. vulgare (X 19).

TABLE I

Species of bacteria	Propyl alcohol Q_{O_2}/N	Isopropyl alcohol Q_{O_2}/N	Glycerol Q_{O_2}/N	Alpha phosphate of glycerol Q_{O_2}/N
E. coli. . . .	30,9	--	846	444
Ps. aeruginosa. .	829	--	690	227
B. vulgare. . . .	171	--	694	497

The experimental conditions were the same as those we describe for the tests on glycerol and its derivatives. The results, as shown on Table I, show that monovalent alcohol with 3 carbon atoms was attacked only if the alcohol group is a primary one. But obviously, as shown in our earlier report, this ability to attack the primary alcohol group is lost in the transition to trivalent alcohols such as glycerol or its esterized product retaining two primary alcohol groups at the ends of the chain (beta phosphates of glycerol), or the product of added acetone (acetone-glycerine) which leaves only a single primary alcohol group free.

In our extension of this research, we were attempting to see whether other monoderivatives of glycerol would behave in the same way as the alpha and beta phosphates of glycerol. What it amounted to was a check as to whether the presence of two kindred oils was sufficient for oxidation, or if the presence of the phosphorus radical was also required.

II. THE EXPERIMENTAL PORTION - For this purpose, we prepared the alpha and beta methyl ethers of glycerol in the pure form, according to a combination of the various existing procedures (Cfr. Aloisi and Bonetti, 1947). As a control, we also chose other substratum substances like ethylic glycol, with two simple kindred oils, the methyl ether of glycol (a single hydroxide), and acetone glycerine (a single free hydroxide). We prepared the methylic ether of ethyl glycol according to the procedure outlined by Cretcher and Pittinger (1947), passing through the ethylene chlorhydrine stage, and the acetone-glycerine according to Fischer and Pfühler (1920). Both the methylic ethers and the phosphoric esters of glycerol, like the glycol correspondents, were tested against the HIO_4 reaction, to check their purity. The alpha phosphate of glycerol contained 88% of pure alpha isomer.

Bacterial species examined:

Bact. paradysenteriae (Flexner), Bact, vulgare (X 19), Diplococcus pneumoniae, Eberthella typhosa, Escherichia coli, Pseudomonas aeruginosa, Serratia marescens, Staph. albus, Acetobacter xylinium (Brown, Bergey et al.), Acetobacter sp. (1)

(1) The strains of Acetobacter were kindly supplied by Professor C. Arnaudi, Director of the Institute of Agrarian Microbiology of the University of Milan, to whom we wish to express our heartfelt thanks.

Except for these last two species, we also checked the phase to which the strains belonged: all were predominately in the S phase for tripaflavin agglutination and according to the morphological characteristics of the colonies.

The germs were cultivated on plates of common agar at 37°C for 18 to 24 hours; Acetobacter xylinium (B.B.) was grown in malt agar, and the Acetobacter sp. in wine agar for 36 to 48 hours at 25°C. The bacterial placque was washed twice in physiological solution, in which there was a final suspension at a concentration corresponding to about 0.25mg of nitrogen per cc (Kjeldahl, repeated several times).

The tests were performed in Warburg respirometers over periods of 2 to 4 hours, with phosphate buffering at pH 7.4, under the conditions outlined above, which provided a resting-phase metabolic rate for the germs tested.

In order to evaluate the significance of the differences between basal respiration and that in the presence of substrata, we tried to establish, within a certain range of error, inherent in the experimental conditions adopted, We achieved this by recording the basal variations in Q_{O_2} for the same suspension of germs, in groups of eleven tests for each germ. Values corresponding to a variation of 3 σ, which includes 99% of the possible error, are shown in the following table which summarizes our results.

TABLE II

Q_{O_2}/N (Consumption of O_2 in mm^3 per mg. of N in bacterial suspension for 1 hour) for the various bacterial species, due to the utilization of the substratum (extra consumption of O_2 in relation to the basal respiration). Experimental conditions: bacterial suspension (about 0.25 mg N/cc; buffer PO_4M/15 at pH 7.4 ccl; substratum M/25cc 0.30; physiological solution to make up the total volume of 3cc; NaOh 10% cc0.20 (in the central reservoir). Gas = air; temperature 38°C. The experiments ranged in length from 2 to 4 hours. In the second to last column at the right are the values corresponding to 3σ calculated on the control tests, with reference to the variations including 99% of the possible errors with the same suspension.

Species of bacteria	Glycerol (OH, OH, OH)	Na, alpha phosphate (OH, OH, P)	Na, beta phosphate (OH, P, OH)	alpha-methyl glycerol (OH, OH, M)	beta-methyl glycerol (OH, M, OH)	ethylene glycol (OH, OH)	methyl glycol (OH, M)	acetone glycerine (OH, A)	Basal respiration	3σ	Length of experiment in hours
B. paradysenteriae (Flexner)	155,5	119,5	17,4	16,4	19,6	7,6	7,1	21,2	54	6,8	2,30
B. vulgare (X 19)	863,-	461,-	28,5	69,2	20,3	5,3	1,-	17,3	55,2	6,7	2,—
D. pneumoniae	4,2	2,8	—	7,2	—	—	1,1	4,9	18	5,6	2,—
E. typhosa	335,-	197,6	29,5	41,5	17,3	5,3	6,-	15,4	44,2	17,4	4,—
E. coli	596,-	269,5	30,7	29,3	20,3	22,1	9,8	19,3	52,7	9,2	2,—
Ps. aeruginosa	728,5	149,5	15,-	48,9	22,5	—	18,8	38,3	107,-	11,8	2,—
S. marcescens	901,-	386,-	21,5	27,4	31,7	1,1	3,4	25,8	131,-	7,3	2,—
St. albus	406,-	183,-	6,4	10,3	—	—	10,6	18,-	56,-	9,-	2,—
Ac. xylinum (B. B.)	37,-	29,9	3,-	8,4	—	—	—	10,2	6,3	—	2,—
Acetobacter sp.	1,8	6	8,3	39,2	3,9	9	9,2	10,2	11,4	—	2,—

III. COMMENT. - In evaluating the data reported here, we consider the most significant to be those contained in the Q_{O_2}/N values greater than 3 . First of all, these results confirm and extend the conclusions of our earlier reports, which indicated that in most of the species examined, there is a very marked consumption of glycerol and its alpha phosphate by oxidation. The highest readings were noted in Bact. paradysenteriae, Bact. vulgare, E. typhosa, E. coli, Ps. aeruginosa and Serratia marcesens. These experiments also proved that in almost every case the consumption of O_2 in the presence of unesterized glycerol is greater than that occasioned by alpha phosphate glycerol.

The beta phosphate of glycerol consistently showed an oxidation rate markedly inferior to that of the alpha isomer, in some cases coinciding with the maximum allowable error deviation.

Interesting by comparison with the data gathered on the phosphoric esters of glycerol are those relating to the Q_{O_2}/N readings on the two methylic ether isomers of the same alcohol. In general, we found low values, although by no means insignificant. They were almost always higher with the alpha methyl-ether than with the corresponding beta isomer, thus providing confirmation, albeit on a very small scale, of the importance of the presence of the two kindred hydroxyls if these molecules are to be subject to germ attack by oxidation.

The major point to be stressed here, however, is that in conjunction with the high consumption rate of O_2 noted in the presence of the alpha ester of glycerol, there is a very low, or sometimes nil utilization of the alpha ether. This goes to show that while the consumption of the glycerol molecule is affected by the presence of kindred hydroxyls, it is, on the other hand, not indifferent to the nature of the radical that links one of the hydrolyls (2), since the methyl radical can never yield the same results as those obtained in the presence of the phosphoric radical.

(2) It is true that in this case we had two compounds belonging to two different chemical classifications; but our knowledge of the product of oxidation allows us to assert that the ester or ether bond has no effect on the type of oxidation that actually occurs, which would, in any case, lead to derivatives of dioxyacetone (Cfr. I. St. Neuberg).

This leads us to support the hypothesis that the glycerol molecule, under the foregoing conditions, is utilized only after phosphorization. This hypothesis is currently being tested in our research activities.

In support of these considerations we can adduce the results of our investigations of other compounds having only free primary hydroxyls: ethylenic glycol, methylic ether, of glycol, and acetone-glycerine.

Ethylenic glycol has two kindred hydroxyls, but as you can see, it cannot be oxidized to any perceptible degree (except by E. coli, which manages to utilize it to a minor extent. Apparently, this is due to the fact that the kinship of the hydroxyls is not completed by phosphorization, and that when the latter does occur, it leads to the loss of the prime condition for oxidation. Glycol's methyl ether (alpha-oxy, beta-methoxytane) behaves in a manner similar to that of the non-etherized compound: it fits into the interpretative pattern we have outlined. Acetone-glycerine, which has only one free hydroxyl, does not, in a general way, behave unlike the pattern described; there is, however, a tendency to yield higher levels for Q_{O_2} as compared with the glycol group, particularly when tested with Ps. aeruginosa. This never yields results higher than those obtained with the alpha methyl glycerol, however. In any case, it is not unlikely that we have here a very slight splitting of the compound during the experiment; on the other hand, it is significant that this molecule shows a very different pattern from those of the simple esters and ethers already considered.

These are the general observations to be made on the basis of our experiments. As for the comparative behavior of the several species, there are obviously major variations, even with the best substrata employed, as a result of the special requirements of the germ. In this way, we have the lively respiration of the less demanding germs (B. vulgare, E. coli, Ps. aeruginosa, and S. marescens), as compared with the really demanding germs such as Diplococcus pneumoniae and Staph. albus; these species give the lowest values (pneumococcus, in our experiments, and under our conditions, failed to attack any of the substrata used). The same thing happened with the two species of Acetobacter, which generally showed a very low respiratory activity, even under experimental conditions specially chosen for these species, and including a pH raised to 6. The almost zero response we found with pneumococci on our substrata bear out the anaerobic experiments with the same germs conducted in 1939 by Jörg, in which he used other substrata in addition to ours. In connection with Acetobacter, it is worthy of note that one of the species we used produced considerable oxidization of the alpha methyl ether of glycol, but was totally inert in relation to the other substrata. Acet. xylinium, on the other hand, showed practically the same effect on glycerine and on its alpha phosphate.

In the literature, I. St. Neuberg (1932) quotes data showing the feasibility of using Acet. suboxydans to transform the alpha methyl ether of glycerol into the monomethylether of dioxyacetone. On the other hand, we find a conflict between our data and those reported earlier by Tosic. He reports observing oxidation of glycerol, and no oxidation of the alpha or beta phosphates, using Acetobacter. (We wonder if the same sp. strain was used.) It is of course true that we are still waiting for a sound and practicable arrangement of Acetobacter.

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